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Prevalence and SEM of *Gnathia* Larvae (Crustacea; Isopoda; Gnathiidae) Infested Marine Fish Species of Two Families, Labridae and Scaridae, from Suez Gulf, Egypt

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ABSTRACT

Gnathiids are external parasites that infest both teleost and cartilaginous fish; they have a developmental stage when they are free-living adults, but they also have a parasitic stage known as praniza larvae, which feed on the host blood. The present study focused on recording the prevalence of parasitic infestations in two families, Labridae (*Coris aygula* with 25% infestation and *Cheilinus lunulatus* with 60% infestation) and Scaridae (*Calotomus viridescense* with 16.66% infestation), collected from El Tor City, South Sinai, Egypt. The *Gnathia* spp. isolated weremostly found attached inside the mouth and beneath the gill arches of fish. Samples were obtained from the host by using forceps from the host and stored in 70% ethanol and 10% formaldehyde for later examinations. The praniza larvae were photographed and identified. The recommendations for later genetic examinations are reported to complete the specific identification and the parasitological map of Suez Gulf fish species.

INTRODUCTION

The Red Sea harbors have a great variety of fish species, rendering it an internationally significant marine environment. The Red Sea, situated in the region between Africa and Asia, provides an optimal environment for fish to flourish due to its elevated temperatures, comprehensive coral reef ecosystems, and abundant biodiversity habitats **(Bogorodsky & Randall, 2019)**. The fish population in the Red Sea is diverse, with a variety of species from different families and ecological niches. The fish in the Red Sea have undergone adaptations to suit the unique environmental circumstances and habitat characteristics, leading to the development of a unique collection of marine organisms **(Golani & Bogorodsky, 2010)**. The presence of temperate tropical aquatic environment and vast coral reefs plays an important role in facilitating the richness and diversity of fish species. The ecosystems of coral reef in the Red Sea, are widely

recognized for their extensive and diverse nature, making them among the most prominent in the world **(Riegl & Purkis, 2012)**. Coral reefs serve as vital ecosystems for fish, providing crucial habitats that give refuge, reproductive sites and food opportunities. The intricate multiple-dimensional configuration of the coral reefs, consisting of sponges, corals and other known benthic animals, generates a varied assortment of micro-habitats capable of sustaining a broad spectrum of fish species.
The Red Sea is a perfect habitat for numerous fish families that are highly prolific as the butterflyfish (family: Chaetodontidae), parrotfish (family: Scaridae), angelfish (family: Pomacanthidae), and wrasses (family: Labridae). Additional species from other families such as Lethrinidae (emperors), Serranidae (groupers), and Acanthuridae (surgeonfish) are commonly recorded **(Golani & Bogorodsky, 2010)**. The fish in the Red Sea are ecologically significanct with their quantity and diversity. Fish are essential for the maintenance of the marine ecosystem's general health and functioning. They have a role in the process of cycling nutrient, regulate smaller creature's populations, and serve as environmental conditions indicators.
Furthermore, specific fish species engage in a symbiotic association with marine corals and other creatures, augmenting the recover and stability of the reef environment **(Alsaaq, 2024)**. The family Labridae, also referred to wrasses fish, is a varied and lively assemblage group of fish that exist in a lot of marine habitats, including seagrass beds, coral reefs, sandy bottoms, and stony coastlines throughout the world **(Khalaf & Kochzius, 2002)**. They display diverse feeding patterns with some species being herbivorous and consuming algae and seagrasses, while others are carnivorous, preying on tiny invertebrates, crustaceans, or even other fish **(Clements & Choat, 2018)**. The Labridae and Scaridae families depend primarily on coral reefs rich in algae. They play important roles in controlling algae growth, participating in cleaning symbiosis, and maintaining ecological balance in reef ecosystems. The diverse feeding behaviors and ecological functions of these organisms are essential for balancing the overall health, adaptability, and development of coral reefs and other dependent marine habitats **(Alwany *et al.*, 2009; Grutter *et al.*, 2020)**.

The Gnathiidae family comprises 12 genera and around 240 species distributed globally **(Aeby *et al.*, 2023)**. This family exhibits differences in sexuality, with adult forms being independent and free-living in sponge cavities, soft seabed tunnels, or coral crevices. The larval forms consist of three phases, each containing two stages: praniza and zuphea. Pranizas are recognized as blood-sucking external parasites on fish; they feed on blood and tissue fluids. Once their gut is fully filled, they descend to the bottom to digest their meal before molting into male or female forms. Pranizas have been primarily reported on the body surface, gills, mouth cavities, and fins of fish **(Ferreira, 2011)**.

The present study discussed the presence of praniza larvae of *Gnathia* spp. in new hosts (Families Labridae and Scaridae) taken from EL Tor City, Suez Gulf in Egypt, with complete morphological characters using scanning electron microscope, focusing on the clearest clinical signs and host-parasite relationship and how it could affect the fish populations in the study location.

MATERIALS AND METHODS

1. Ethical statement

The following protocols involving animals were established according to the criteria of guidelines of the Ethical Animal Use in the Research Committee (EAURC), Faculty of Science, Suez Canal University, Egypt (Approved with number, REC346/2024).

2. Fish collection and species identification

A total number of 200 specimens of families Labridae (140 specimens) and Scaridae (60 specimen) were collected from fishermen in the fish markets of El-Tor City from South Sinai, located in Suez Gulf, Egypt during the summer of 2023. Fish were obtained either alive or newly deceased and transported to the Aquatic Pathology Laboratory at National Institute of Oceanography and Fisheries (NIOF), Suez and El-Aqaba branch, Egypt, to complete parasitological examinations. Fish species were identified according to the international FISHBASE, the largest database of fishes on the global level, Which was also used to confirm the information collected on each species **(Farghal *et al.*, 2021)**.

3. Clinical signs and postmortem examinations

Fish specimens were clinically assessed for external signs and post-mortem abnormalities according to the methods of **Conroy and Herman (1970)**.

4. Parasitological examinations

Fish specimens were examined externally under a dissecting light microscope, and then each fish was opened to examine the organs separately. Isopod larvae were cleaned with a 0.5% saline solution and then fixed in 70% alcohol. Gnathiid parasites were identified using the identification keys provided by **Yamaguti (1971)**, **Paperna (1996)** and **Hoffman (2019)**.

5. Dissecting stereo-microscope examinations

Gnathia larvae were isolated and examined then photographed by dissecting stereo- and light microscopes (OPTICA B-150, Italy) according to **Nashaat *et al.* (2023)**.

6. Scanning electron microscope (SEM)

The parasites were transported to the Electron Microscopy Unit (EMU) at Assiut University, Egypt, where the scanning electron microscope (SEM) process was completed. Samples were repeatedly rinsed in isotonic saline solution and were forcefully agitated to dislodge and remove any adhering debris. They were then washed with phosphate buffer solution and fixed in 3% glutaraldehyde, as described by **Colwell *et al.* (2007)**. All samples were dehydrated using liquid carbon dioxide and mounted on metallic blocks with silver paint. A gold sputter coater was used to apply a 15nm thick layer of gold to the samples. They were then analyzed and imaged using scanning electron microscopy (JEOL JSM 5400 LV, England) at magnifications of 15–25 kV, following the methods described by **Bozzola and Russell (1999)**.

RESULTS

1. Fish taxonomy

Table 1. The classification of the fish collected

Species	***Coris aygula*** **(Lacepède, 1801)**	***Cheilinus lunulatus*** **(Forsskål in Niebuhr, 1775)**	***Calotomus viridescens*** **(Ruppell, 1835)**
Class	Actinopterygii	Actinopterygii	Actinopterygii
Family	Labridae	Labridae	Scaridae
Genus	*Coris*	*Cheilinus*	*Calotomus*
Common name	Clown Coris Wrasse	Broomtail Wrasses	Parrotfish

*The common name means the fish name in the markets

2. Clinical signs of samples collected

Clinical signs related to parasitic infestations detected from infested fish revealed light to moderate pathogenic signs such as opened mouth and discolored gills with bulging eyes and also mucus accumulation on the body surface due to the presence of the larval Gnathiidae attached inside the mouth cavity and beneath the gill arches (plate1).

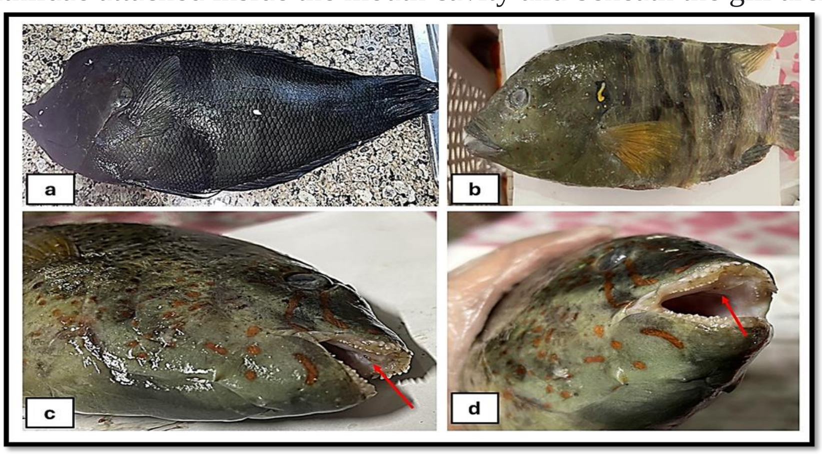

Plate 1. Species of families Labridae and Scaridae **(a)** *Coris aygula* (Lacepède, 1801) showing excess mucus secretions, **(b)** *Cheilinus lunulatus* (Forsskål in Niebuhr, 1775) showing bulging eye, **(c and d)** *Calotomus viridescens* (Ruppell, 1835) with attached Gnathiidae larvae inside the mouth cavity

3. Parasite taxonomy

Order: Isopoda
Suborder: *Cymothoida Wägele*, **1989**
Superfamily: *Cymothooidea Leach*, **1814**
Family: *Gnathiidae Leach*, **1814**
Species: *Gnathia* sp.

4. Prevalence of parasitic infestation

As shown in Table (2), the total number of examined fish from the Labridae family revealed that out of 40 samples of *Coris aygula*, 25% were infested with Gnathiidae larvae, while 75% were non-infested. In the case of *Cheilinus lunulatus*, 100 samples were examined, showing that 60% were infested and 40% were non-infested. For *Calotomus viridescens* from the Scaridae family, 60 samples were examined, indicating a 16.66% infestation rate and 83.33% non-infested.

Table 2. Prevalence of parasitic infestations of *C. aygula*, *C. lunulatus* and *C. viridescense*

Fish species	Total examined	prevalence	Infested	Prevalence	Non-infested	Prevalence
Coris aygula	40	20%	10	25%	30	75%
Cheilinus lunulatus	100	50%	60	60%	40	40%
Calotomus viridescense	60	30%	10	16.66%	50	83.33%
Total	200	100%	80	40%	120	60%

Prevalence: Percentage of the infested fish from the total examined ones.

5. Morphological characters of *Gnathia* spp. (praniza larvae)

The morphological description of the larvae shows that the body is elongated (2 ± 0.5 mm, n = 10), featuring an enlarged cephalosome with small concave margins and a few sensory pits on the dorsal surface. The anterior margins of the cephalosome are straight, with lateral excavations that expand the first part of the antennae. The antennule is well-defined and shorter than the antenna. The oval-shaped eyes are well-developed, darkly colored, and positioned on both sides of the cephalosome. The first pereonite is partially attached to the cephalosome, while the second and third pereonites are similar in shape and size. The fourth pereonite is triangular and expands over the fifth, with lateral leg attachments. The sixth pereonite is rectangular with slightly concave margins, also featuring lateral leg attachments. The seventh pereonite is rounded and connects with the first pleon. Both the pleon and pleotelson are elongated, with overlapping concave margins. The uropod rami extend to the apex of the pleotelson, which is narrowly rounded (for a detailed description, see Plates 2 and 3).

As shown in Plate (2), photographs of praniza larvae collected from Labridae and Scaridae fishes were preserved in 70% alcohol for later examination using light and scanning electron microscopy (photos taken with an iPhone 12 Pro Max, 2.5 mm). Panel (a) shows the whole larvae, capturing the body parts using a light microscope at 40X magnification (panels b and c). Some fresh larvae, filled with blood meal, appear with red abdomens (photo taken with an iPhone 12 Pro Max, 2.5 mm) (panel d). Panel (e) features a light microscope photo of fresh samples of the pleotelson and uropods, highlighting the segments, as well as the cephalosome (head region) with enlarged, darkly colored eyes (40X).

Plate 2. Photos of fresh praniza larvae of *Gnathia* spp.

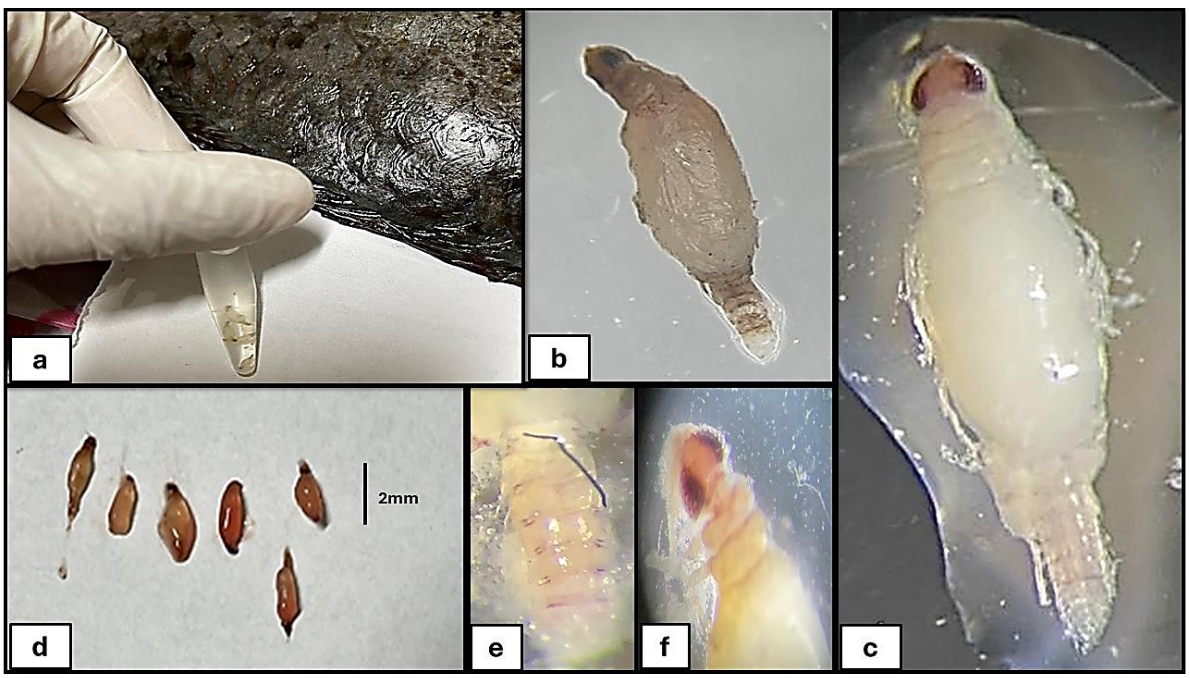

Plate 2.(a) Collected samples of praniza larvae isolated from Labridae and Scaridae fishes stored in preservative. **(b and c)** Whole fresh sample showing body parts (head, abdomen and pleotelson) (40X). **(d)** Freshly isolated praniza larvae showing some filled with host blood meal (with red color) 2mm. **(e)** Uropods and pleotelson showing segments (40X). **(f)** Cephalosome with elongated eyes (head region) (40X)

6. Scanning electron microscope findings (SEM)

As shown in Plate (3), the ventral view of the whole body of praniza larvae of *Gnathia* spp. is presented (a), with a scale bar of 1 mm, indicating that the parasite is visible to the naked eye and can be easily isolated from infested fish. Praniza larvae are characterized by enlarged uropods and pleotelson (b), with a scale of 500µm. The cephalosome features partially fused pereonites dorsally, along with well-developed oval-shaped eyes on both sides of the anterior body, accompanied by attached antennae and shorter antennules (c, d, and e), with a scale of 500-100µm. The triangular-shaped pereiopods have penicillate setae scattered across all appendages, with a lateral shield at the leg attachment (f), scale 100µm. The pleon, with all pleonites visible ventrally, is slightly larger than the pereonites, and the pleotelson is usually covered with pectinate scales, with the apex exhibiting two setae (g, h, and i), scale 100-50µm.

Plate 3. SEM of praniza larvae of *Gnathia* spp.

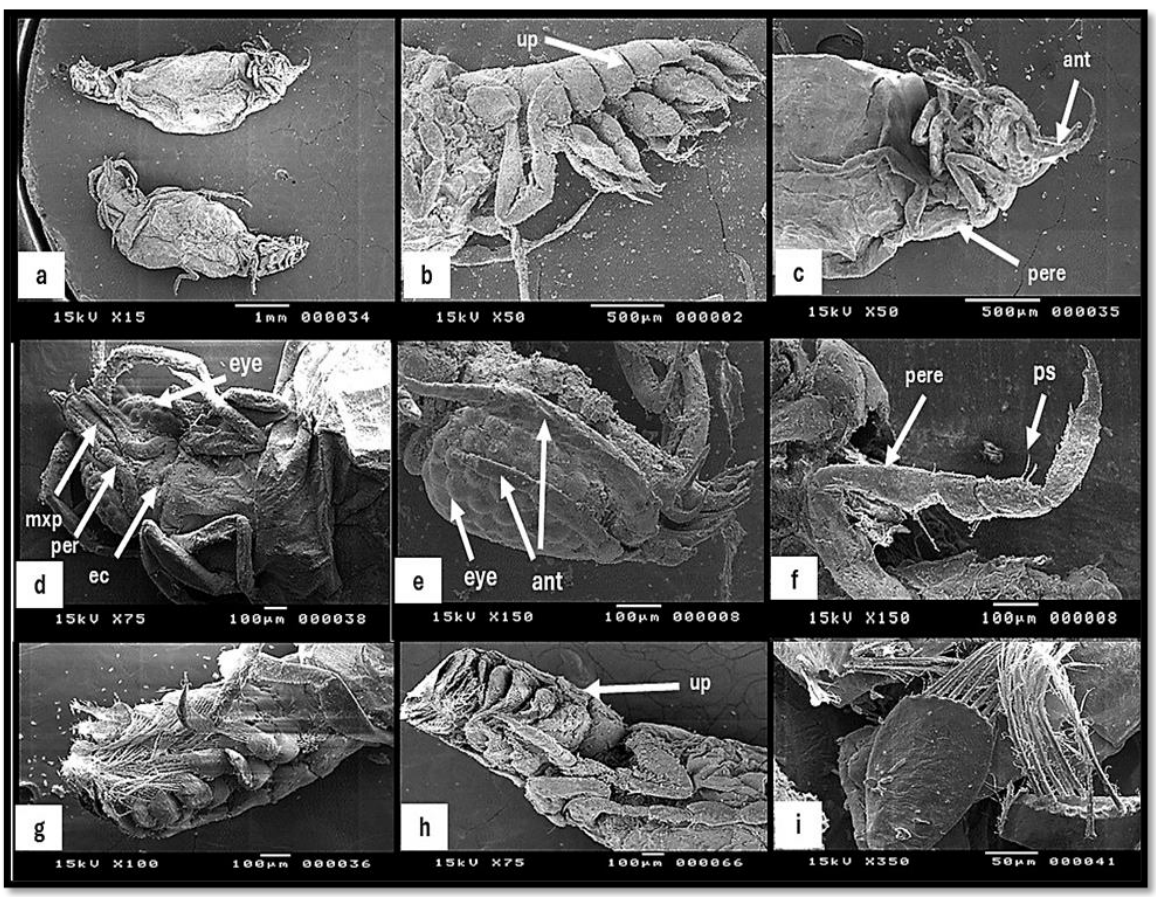

Plate 3. Praniza larvae of *Gnathia* spp. Isopods showing: **(a)** General view of the ventral side of Gnathiidae larvae 1mm. **(b)** Enlargement of pleotelson and uropods (up) with walking appendages 500µm. **(c)** and **(d)** Ventral view of cephalosome showing mouthparts with antenna (ant), shorter antennule, 1st pereonite (per) attached ventrally with cephalosome, maxilliped (mxp) cylindrical with elongated base attached palp margins, membranous cuticle of pereonite (ec) and

enlarged eye, 500-100µm. **(e)** lateral view of enlarged eye with antenna and antennule (ant)100µm. **(f)** Pereiopods (pere) and penicillate seta (ps) 100µm. **(g)** and **(h)** Enlarged pleotelson and uropods 100µm. **(i)** Dorsal view of pylopods 50µm

DISCUSSION

Parasitism of marine fishes are considered a serious problem nowadays affecting the wild population **(Bayoumy *et al.*, 2013)**. Numerous fish species are parasitized by isopods in different habitats, which causes significant economic losses **(Youssef *et al.*, 2018)**. Gnathia larvae are widely distributed parasitic species affecting marine fish **(Aguilar-Perera, 2022)**.

Praniza larvae of *Gnathia* spp. are common ectoparasites of cartilaginous and teleost fishes, characterized by low host specificity and distribution across various habitats. They feed on the blood of their hosts, leading to severe tissue damage **(Ayari, 2004)**. Clinical examinations of infected fish revealed clear signs of infestation, such as excess mucus secretions, bulging eyes, emaciation, inflammation on the body surface, and damaged tissue at attachment sites. These effects are consistent with the blood-feeding nature of this parasite, as noted by **Martens and Moens (1995)**. **Zayed *et al.* (2023)** also stated that *Gnathia* spp. are widely dispersed among various hosts, causing significant injury in different parts of the host's body.

The present study revealed that parasitism by *Gnathia* spp. larvae occurred in two Labridae species (*Coris aygula* 25% and *Cheilinus lunulatus* 60%) and one Scaridae species (*Calotomus viridescens* 16.66%). This aligns with the findings of **Zayed *et al.* (2023)**, who isolated *Gnathia* spp. larvae from different fish species with varying prevalence rates: *Dicentrarchus labrax* (8.1%), *Mugil cephalus* (12.6%), *Sparus aurata* (13.7%), *Mullus surmuletus* (8.9%), *Lithognathus mormyrus* (14.4%), and *Pagellus erythrinus* (17.7%) from the Egyptian coast near Alexandria, all of which are lower than the rates found in this study. Similarly, **Bayoumy *et al.* (2013)** reported a prevalence of 58.33% for *Gnathia* spp. larvae in the Arabian Gulf greasy grouper (*Epinephelus tauvina*) caught in Saudi coastal waters.

Öktener and Tuncer (2020) documented *Gnathia* larvae infestations in *Sargocentron rabrum*, *Parapenus forskali*, and *Upeneus moluccensis* along the coast of Turkey in the Aegean Sea, with prevalence rates of 58, 63, and 47%, respectively. Additionally, **Adday and Khamees (2022)** found a 69% prevalence of *Gnathia* spp. larvae in the gill lamellae of *Chiloscyllium arabicum* in Iraqi coastal waters. In the current study, the primary sites of isolated praniza larvae were the buccal cavity and beneath the gill chambers of all examined samples. **Katsuhiko and Masakazu (2000)** noted that praniza larvae typically remain in the gills and gill cavities for one or more days, as these areas are rich in blood supply from the gill arches and filaments.

Gnathiid isopods are usually identified based on mature males obtained through techniques such as elutriation, dredge sampling, sorting of coral rubble, and sponge collections. Since the twentieth century, researchers have identified new species of *Gnathiids* by observing the transformation of the last larval stages into adults. This has been achieved through various methods, including using light to attract specimens **(Farquharson *et al.*, 2012)**, employing baited traps **(Shodipo *et al.*, 2021)**, or collecting them directly from their hosts. The morphological characterization of praniza larvae showed an enlarged cephalosome with well-developed eyes. These features, along with others described in the results, demonstrate similarities among most *Gnathiid* species, as noted by **Smit and Hadfield (2022)** in their description of *Gnathia pipinde* isolated from the pufferfish (*Amblyrhynchotes honckenii*) in South Africa. **Kumaş *et al.* (2024)** also described *Gnathia* spp. larvae isolated from *Labrus viridis* collected in the Aegean Sea, Turkey. The isolated praniza larvae exhibited different colors depending on the amount of blood ingested from their host.

The Gnathiidae family comprises 12 genera and an estimated 240 species distributed globally **(Erasmus *et al.*, 2023)**. Therefore, specific classification should rely not only on morphological characteristics but also on genetic identification.

CONCLUSION

In this study, we recommend complementing the specific identification of the detected praniza larvae of *Gnathia* species with molecular methods. Additionally, we suggest discussing the host-parasitic relationships and their ecological effects on the environment in the Suez Gulf region to better understand the impact of parasitism on fish populations in the study area.

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